Original Article

Effects of Acute Bouts of Swimming Exercise on AMPKα2 Expression in Mouse Skeletal Muscle

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The 5′-AMP-activated protein kinase (AMPK) is a multisubstrate serine/threonine protein kinase that is ubiquitously expressed and functions as an intracellular fuel sensor activated by depletion of high energy phosphate compounds. The α2 isoform is by far the most abundant in skeletal muscle, representing at least 66% of total AMPK activity. The purpose of this study was to determine the effects of the different exercise (swimming) durations on AMPKα2 gene and protein expression, and the relationships between the expression of AMPK and blood glucose and muscle glycogen. C57 mice were divided into three groups: a sedentary control group, a 90-minute swimming exercise group, and an exhaustive swimming exercise group. AMPKα2 gene and protein expression in the quadriceps muscle were measured separately by real-time quantitative PCR and Western immunoblot after the swimming exercise. Blood glucose and muscle glycogen were also measured at the same time. The results showed that 90-minute and exhaustive swimming exercise induced significant increases in skeletal muscle AMPKα2 gene and protein expression compared to in the control group, and AMPKα2 expression was higher after exhaustive swimming than after 90-minute swimming exercise. There was a significant negative correlation between AMPKα2 expression and blood glucose level. The high AMPKα2 gene and protein expression induced by exercise might be one of the reasons for blood glucose reduction, but not the key factor which regulates the degradation of muscle glycogen during swimming exercise. [*J Exerc Sci Fit* • Vol 8 • No 1 • 11–16 • 2010]

Keywords: AMPKα2 expression, blood glucose, mice, muscle glycogen, swimming exercise

Introduction

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase that responds to cellular energy status by inhibiting ATP-consuming pathways and activating ATP-producing pathways (Hardie & Sakamoto 2006; Aschenbach et al. 2004), and is active as a heterotrimer consisting of one catalytic subunit (α) and two non-catalytic subunits (β, γ) (Hardie 2004). The α2 isoform is by far the most abundant in skeletal muscle, representing at least 66% of total AMPK activity (Viollet et al. 2003). In skeletal muscle, the activity of AMPK is thought to be mediated mainly through the AMPKα2 isoform (Wojtaszewski et al. 2002; Musi et al. 2001b).

The majority of human studies show that AMPK is activated in an exercise-intensity-dependent manner and there is a more pronounced response of AMPKα2 activation compared with AMPKα1 (Chen et al. 2003; Wojtaszewski et al. 2000). For example, Fujii et al. (2000) reported that AMPKα2 activity was significantly increased in vastus lateralis biopsy samples following 60 minutes of cycle ergometry at 70% $\dot{V}O_{2peak}$ but not at 50% $\dot{V}O_{2peak}$. No changes in α1 activity were observed at either exercise intensity (Fujii et al. 2000). In mice, it was reported that after 30 minutes of moderate-intensity swimming exercise, AMPKα2 activity increased by 80%, but AMPKα1 activity did not change significantly in the hind limb muscles (Nakano et al. 2006).

A few studies attempted to address the relation between exercise duration and activity of AMPKα2, and

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found that when exercise time is prolonged, AMPKα2 activity is increased correspondingly (Lee-Young et al. 2006; Stephens et al. 2002; Wojtaszewski et al. 2002). However, in most of the studies, only the activity of AMPK was measured during acute bouts of exercise and it remains unclear how different exercise times affect AMPKα2 gene and protein expression.

The purpose of this study was to determine the effects of different exercise (swimming) times on AMPKα2 gene and protein expression, and the relationships between AMPK expression and blood glucose and muscle glycogen.

Methods

Animal characteristics

All experiments were approved by the Beijing Sport University animal experiments inspectorate committee. Female C57 mice (30–35 g) were purchased from Peking University. The animals were housed in rooms with light from 7 AM to 7 PM and were allowed free access to water and standard chow. Room temperature was maintained at 20–22°C.

Exercise protocol

The mice were randomly divided into three groups ($n = 8$ in each group): a sedentary control group, a 90-minute swimming exercise group, and an exhaustive swimming exercise group. Mice in the swimming groups were subjected to swim in a barrel with a swimming area of 20 cm^2 per mouse. The mean swim-to-exhaustion time was 2.75 hours in this study. The assessment of swim-to-exhaustion was done as previously described (Thomas & Marshall 1988). The water temperature was maintained at 32 ± 2°C during all swimming exercises.

Muscle lysate preparation

Approximately 70 mg of freeze-dried quadriceps muscle tissue was homogenized with buffer 1 [50 mM Tris-HCl (pH 7.4–8.0), 150 mM NaCl, 5 mM EDTA, 2 mM PMSF (phenylmethylsulfonyl fluoride), 1% Triton X-100], and let stand for 15 minutes on ice, then centrifuged for 60 minutes (12,000 rpm, 4°C). The supernatant was harvested, frozen in liquid nitrogen and stored at –80°C. Total protein content in the lysate was determined using the Bradford method (Bradford 1976).

Western blotting

About 30 μg of muscle lysate proteins were separated with the use of standard SDS-PAGE (5% stacking gel, 10% running gel) and transferred to nitrocellulose filter membranes (Bio-Rad Laboratories, Hercules, CA, USA) by wet transfer (250 mA, 90 minutes). Membranes were blocked in Tris-buffered saline containing 5% skimmed milk protein for 20 minutes at room temperature, then incubated with primary antibodies (AMPKα2 goat polyclonal IgG; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C, followed by incubation with horseradish peroxidase-conjugated secondary antibody (donkey anti-goat immunoglobulins, horseradish peroxidase-conjugated; Santa Cruz Biotechnology) and the housekeeping gene β-actin (Santa Cruz Biotechnology) for 1 hour at room temperature. Bands were visualized using a Kodak Image Station 440CF (Kodak, Rochester, NY, USA) and enhanced chemoluminescence (Pierce, Rockford, IL, USA), and were quantified using Quantity One software. The protein content was expressed in relative units in comparison with control samples loaded on each gel.

RNA isolation and RT-PCR

Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA was quantitated as previously described (Nicot et al. 2005). Oligo dT single-stranded cDNA was synthesized using the ImProm-II Reverse Transcriptase kit (Promega, Madison, WI, USA). Forward and reverse primers complementary to the mouse AMPKα2 gene (GeneID: 108079) were designed using Primer Premier 5.0 software (PRIMER Biosoft International, Palo Alto, CA, USA). The AMPKα2 forward primer sequence (5′ to 3′) was GAT GAT GAG GTG GTG GA, while the reverse primer sequence was GCC GAG GAC AAA GTG C.

Real-time PCR was performed using SYBR Green PCR Reagents on ABI One-Step Detection System (Applied Biosystems, Foster City, CA, USA). Ten ng of cDNA were added and 300 nM each for specific forward and reverse primers were used. The following amplification program was used: 94°C for 10 minutes, 40 cycles at 94°C for 30 seconds followed by 60°C for 1 minute. All samples were amplified in triplicate from the same RNA preparation and the mean value was considered. Changes in AMPKα2 gene expression was normalized to the housekeeping gene β-actin and AMPKα2 mRNA was expressed as a fold expression relative to the mean values observed in each control group.

Muscle glycogen and blood glucose content

Quadriceps muscle glycogen content was determined as originally described by Lowry and Passonneau (1972),

and blood glucose level was obtained by the oxidase method (HITACHI 7600; Hitachi, Tokyo, Japan).

Statistical analysis

All values are expressed as mean ± standard deviation. Statistical comparisons were made by one-way ANOVA and correlation analysis was performed by Pearson product moment using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $p < 0.01$.

Results

Effects of different exercise times on AMPKα2 mRNA levels and protein expression in skeletal muscle

AMPKα2 mRNA level increased by 50.8% relative to the control group ($p < 0.01$) after 90 minutes of moderate-intensity swimming exercise, and increased by 88.2% after exhaustive exercise (Figure 1). Similarly, AMPKα2 protein expression increased by 1.4- and 2.1-folds relative to the control group ($p < 0.01$) after 90 minutes and exhaustive swimming exercises, respectively (Figure 2).

Effects of different exercise times on muscle glycogen and blood glucose levels

Blood glucose concentrations were measured in all groups, and those of the 90-minute and exhaustive exercise groups significantly decreased by 55.4% and 72.8%, respectively, compared with the control group ($p < 0.01$). Moreover, the blood glucose concentration in the exhaustive exercise group was lower by 39.1% compared with the 90-minute exercise group (Figure 3).

Swimming exercise reduced ($p < 0.01$, main effect) muscle glycogen content by 36% and 50% in the exhaustion and 90-minute groups, respectively (Figure 4). There were no significant differences between the exhaustion and 90-minute low-intensity prolonged swimming exercise groups.

Relationship between AMPKα2 expression and blood glucose level

As shown in Figures 5 and 6, the exercise-induced decrease in blood glucose correlated significantly with measures of exercise-induced changes in AMPKα2 expression. The correlations between AMPKα2 expression and muscle glycogen were not significant (not shown).

Discussion

A number of studies have shown that AMPK is activated in skeletal muscle during exercise in an

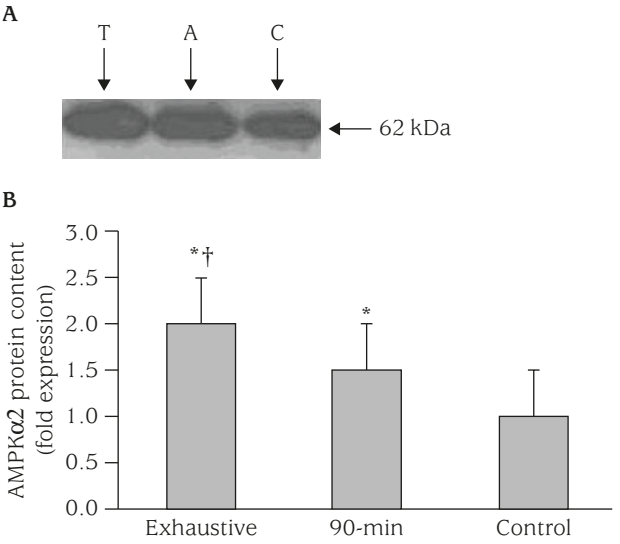

Fig. 2 Western blot for AMPKα2 protein expression in the skeletal muscle of the three groups of mice. *$p < 0.01$ *vs.* control group; †$p < 0.01$ *vs.* 90-minute exercise group. T = exhaustive exercise group; A = 90-minute exercise group; C = control group.

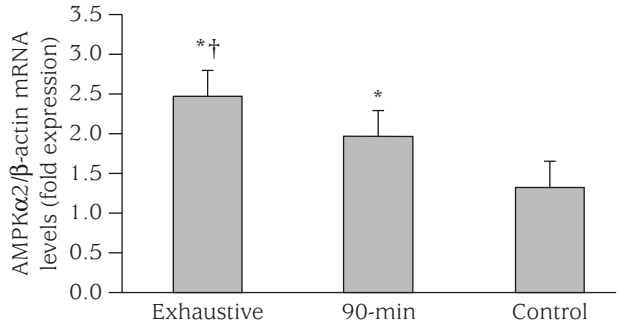

Fig. 1 AMPKα2 mRNA levels in the skeletal muscle of the three groups of mice. *$p < 0.01$ *vs.* control group; †$p < 0.01$ *vs.* 90-minute exercise group.

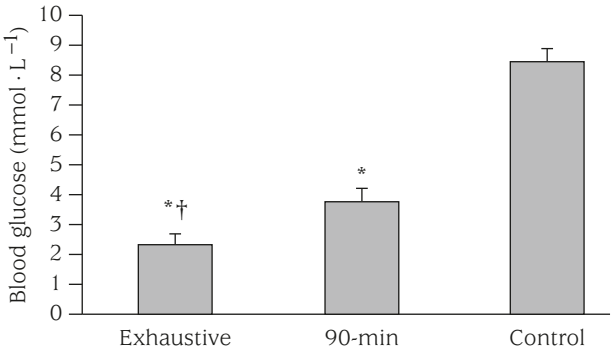

Fig. 3 Blood glucose levels in the three groups of mice. *$p < 0.01$ *vs.* control group; †$p < 0.01$ *vs.* 90-minute exercise group.

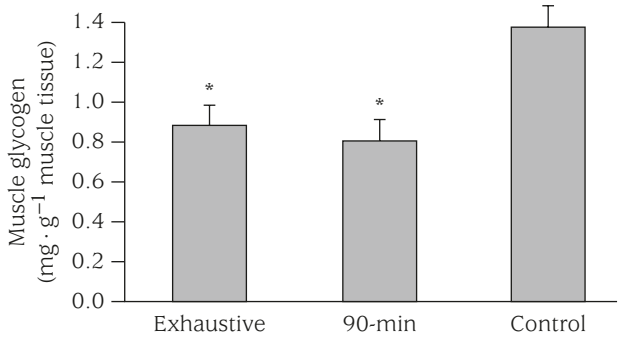

Fig. 4 Muscle glycogen content in the three groups of mice. *$p < 0.01$ *vs.* control group.

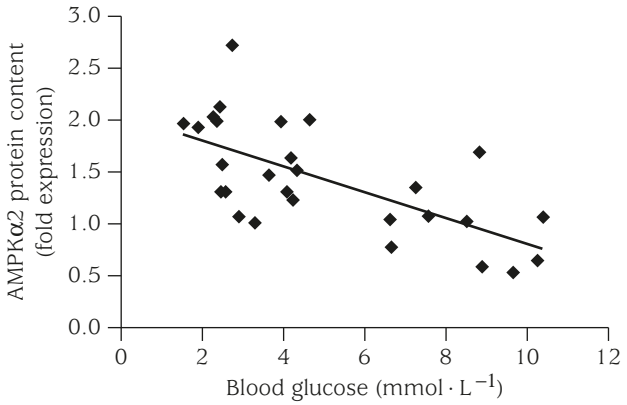

Fig. 6. Scatter plot of AMPKα2 protein content and blood glucose ($r = -0.67$, $p < 0.01$).

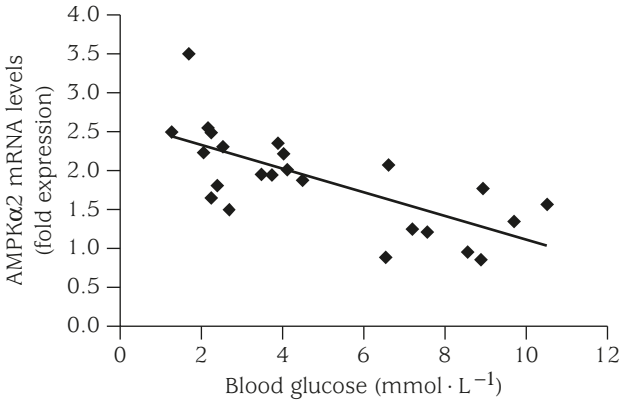

Fig. 5 Scatter plot of AMPKα2 mRNA and blood glucose ($r = -0.69$, $p < 0.01$).

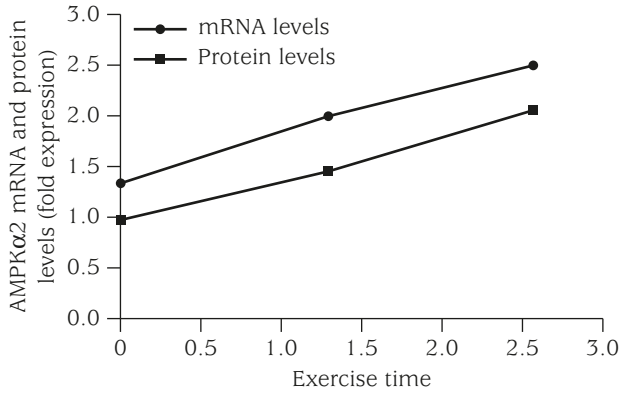

Fig. 7 Changes in AMPKα2 expression in skeletal muscle with time.

exercise-intensity-dependent manner (Chen et al. 2003; Wojtaszewski et al. 2000). To investigate the relationship between exercise time and AMPKα2 expression, we measured AMPKα2 expression after different durations of exercise. After prolonged exercise, AMPKα2 signaling was increased compared with the control group. This is in accordance with the findings of previous studies (Lee-Young et al. 2006; Stephens et al. 2002; Wojtaszewski et al. 2002; Musi et al. 2001a). Stephens et al. (2002) investigated seven individuals who cycled for 30 minutes at a workload requiring 62.8 ± 1.3% $\dot{V}O_{2peak}$ and obtained their vastus lateralis with muscle biopsies at rest and at 5 and 30 minutes of exercise. They found that AMPKα2 activity was significantly elevated after 5 minutes (~2-fold), and further elevated after 30 minutes (~3-fold) of exercise. Other studies found similar results. Musi et al (2001a) showed that during 45 minutes of cycle exercise, AMPKα2 activity was 2.7-fold of basal activity at 20 minutes and remained elevated throughout the protocol. Lee-Young et al. (2006) found that during 2-hour exercise, AMPKα2 activity showed a continuous increase.

However, most of these studies only measured AMPKα2 activity, and few involved protein and gene expression. Our study found that different durations of swimming exercise increased AMPKα2 gene and protein expression in skeletal muscle (Figures 1 and 2), and its expression appeared to be time-dependent (Figure 7). The changes in AMPKα2 gene and protein expression were similar to the changes in AMPKα2 activity mentioned above.

The metabolic actions of AMPK have been extensively studied in skeletal muscle. Activation of AMPK by 5-amino-4-imidazolecarboxamide-riboside (AICAR) has been demonstrated to increase muscle glucose uptake *in vivo* and *in vitro* via an acute insulin-independent mechanism (Mu et al. 2001; Bergeron et al. 1999). *In vitro* AICAR-incubated muscles from transgenic mice where

reduction of AMPK signaling by either overexpressing a kinase-dead AMPKα2 or knocking out the catalytic α2 isoform could completely abolish AICAR-stimulated glucose uptake (Barnes et al. 2004; Jørgensen et al. 2004). In the current study, there was a significant decrease in blood glucose concentration after the acute exercises (Figure 3), and the changes were associated with a pronounced increase in AMPKα2 gene and protein expression in skeletal muscle (Figures 6 and 7). The high AMPKα2 gene and protein expression induced by exercise might be one of the main reasons for blood glucose reduction.

Glycogen is an important energy source for the working muscle, especially at moderate to high intensities of exercise, and it is broken down by glycogen phosphorylase (GP) and glycogen disbranching enzyme to provide glycosyl units for glycolysis and oxidative phosphorylation. After a bout of exercise, or in response to insulin, glycogen is under the consumption of ATP (re)synthesized from cellular glucose by a chain of enzymes, and the incorporation of UDP-glucose to glycogen by glycogen synthase is believed to be the rate-limiting step (Roach 2002). Regulation of GP and glycogen synthase is complex and depends on both allosteric and covalent mechanisms. Originally, GP-kinase was found to be a target for AMPK, potentially enhancing GP activity and glycogenolysis (Young et al. 1997; Carling et al. 1989). However, more recent *in vitro* evidence has shown that GP-kinase is unlikely to be an AMPK substrate, suggesting that AMPK does not regulate glycogenolysis (Beyer et al. 2000). The finding that glycogen breakdown during treadmill running in muscle from either the AMPK kinase-dead mouse or the AMPKα2 knockout mouse is not compromised (Jørgensen et al. 2005; Mu et al. 2003) could support such a view. Thus, in our study, there was a significant decrease in quadriceps muscle glycogen content in the 90-minute swimming exercise and exhaustive swimming exercise groups compared with the control group (Figure 4), and no significant correlation between AMPKα2 expression and muscle glycogen content. That may suggest that high AMPKα2 expression is not the key factor that regulates the degradation of muscle glycogen during swimming exercise.

Conclusion

The 90-minute and exhaustive swimming exercises induced a significant increase in skeletal muscle AMPKα2 gene and protein expression compared with the control group. AMPKα2 expression was higher after exhaustive swimming than after the 90-minute swimming exercise, indicating that AMPKα2 expression is time-dependent. There was a significant negative correlation between AMPKα2 expression and blood glucose level. The high AMPKα2 gene and protein expression induced by exercise might be one of the reasons for blood glucose reduction, but not the key factor that regulates the degradation of muscle glycogen during swimming exercise.

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